CLINICAL STUDY

Clinical Relevance of Cryglobulinaemia and Extrahepatic Neurocutaneous Manifestations of Chronic Hepatitis C

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Abstract

Objective

To examine the prevalence of cryoglobulinaemia in patients with chronic hepatitis C infection and its relation to extrahepatic neuro-cutaneous manifestations.

Methods

Forty patients (26 males & 14 females), with clinical, laboratory and histologically established chronic hepatitis C infection, with a mean age of 37.5 years, were submitted to clinical examination, dermatological and neurological evaluation. Neuroimaging as well as neurophysiological evaluation, laboratory assessment including liver function tests, serum cryoprecipitate immunoelectrophoresis, and revision of histopathlogical findings were performed.

Results

A high prevalence of cryglobulenemia: 62.5% in patients with chronic hepatitis C infection, the presenting symptoms were fatigue (67.5%), arthralgia (32.5%), paresthesia (30%) and pruritus (25%); however, there were no statistically significant difference between cryo +ve versus cryo –ve patients except for pruritis, and face pigmentation. Skin manifestations including face pigmentation (42.5%), leukocytoclastic vasculitis (22.5%), porphyria cutanea tarda (20%), lichen planus (17.5%), acral necrolytic erythema (15%) and vitiligo (15%). Neurological manifestations ; symptomatic neuropathy in 10%, neuropathic changes in 30% and electroencephalographic changes in 22.5%. These cutaneous and neurological manifestations were significantly associated with the presence of cryoglobulinaemia.

Conclusion

Our findings support an association between cryoglobuliaemia and extrahepatic neurocutaneous manifestations of hepatitis C infection. The presence of all these manifestations in the appropriate clinical setting should suggest the presence of hepatitis C infection.

Key Words: Chronic hepatitis C, cryoglobulinaemia, extrahepatic neurocutaneous manifestations

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Introduction

Nowadays, there is an increasing awareness of a variety of extrahepatic syndromes that seem to be associated with HCV infection[1].

Pascual *et al*[2] reported two patients with chronic HCV infection and essential mixed cryoglobulinaemia (EMC). Since then, a considerable number of extrahepatic syndromes has been reported as described by Nocente *et al*[3].

Cryoglobulinaemia (Cg) is a condition characterized by the presence of serum proteins that reversibly precipitate in cold[4] and are classified on the basis of their immunoglobulin composition according to Brouet *et al*[5]. The clinical manifestations of mixed cryoglobulinaemia (MC) range from asymptomatic, mild vasculitis with palpable purpura, arthralgia and fatigue, to severe vasculitis with skin necrosis[6], involvement of the kidneys[7], peripheral nerves[8], and centeral nervous system[9]. Fifty percent of patients with chronic HCV, show detectable cryoglobulinaemia, even though most of them do not show cryoglobulinaemia related symptom[10]. Factors involved in the production of Cg in HCV infected patients are unknown; most likely the presence of HCV in the cells of the immune system and /or chronic stimulation of the immune response by HCV. Also, genetic factors may be involved in the pathogenesis of Cg. Little is known about the clinical significance of Cg in the course of chronic HCV[11].

Neurological manifestations of HCV have been less well characterized and reported, including peripheral nerve vasculitic neuropathy, and also intracranial vasculopathy[12,13]. Peripheral neuropathy is present in most of the patients with symptomatic cryoglobulinaemia where it may be the first clinical manifestations[10]. In 1998, Origgi and Colleagues[14], described 3 patients who complained of mild CNS problems, including confusion and disequilibrium and magnatic resonance imaging (MRI) of these patients showed small hyperintensities consistant with ischaemic lesions. In Heckmann et al[9]. review of a small series of HCV-infected patients showing neurological manifestations, a patient with systemic vasculitis associated with systemic cryoglobulinaemia is described who progressed from apathy to severe drowsiness with myoclonic jerks and multiple pyramidal tract signs and MRI showed increased prolonged Spin echo signal diffusely throughout the deep white matter that resemble HIV-associated leukoencephalopathy.

Chronic HCV is associated with and may trigger or exacerbate an extraordinary variety of extrahepatic skin manifestations, the most frequent and important of which are the leukocytoclastic vasculitis (LCV) of mixed cryoglobulinaemia type 2 and porphyria cutinea tarda (PCT)[15]. Cutaneous symptoms relevant to HCV infection manifest in 20 – 40 % of patients presenting to dermatologists and in a significant percentage (15 – 20 %) of general patients and HCV are suggested and must appear in the differential diagnosis of these patients to avoid missing this important but occult factor in clinical disease in the appropriate setting[16]. Leukocytoclastic vasculitis (LCV) due to cryoglobulinaemia is a good example of a specific skin manifestation resulting from the production of immunoglobulins with rheumatoid factor characteristics causing an immune complex- mediated vasculitis while Porpheria cutanea tarda (PCT) is a good example of HCV-related disease in which causation is undeniable where 70 % of patients with PCT have HCV[16]. The objective of this work was to determine the prevalence of Cg in our patients with chronic HCV infection and its relation to the neuro-cutaneous extrahepatic manifestations.

Materials and Methods

Forty patients (26 males and 14 females), with established diagnosis of chronic HCV (clinical, laboratory and histopathological), with a mean age of 37.5 years were studied. Exclusion criteria were the following:

1. Chronic hepatitis C patients with Fibrosis score (F) beyond F1
2. Other liver diseases including the

schistosomiasis, hepatitis B, autoimmune or drug-induced hepatitis.
3.The presence of other causes of cryoglobulinaemia including HIV, malignancy, or other chronic diseases .
4. Previous antiviral therapy in the patient population before their assessment.
Patients were selected from patients who were evaluated for antiviral therapy at the Clinic of Gastroenterology and Hepatology of Specialized Medical Hospital Mansoura University during January 2004 to January 2006. Patients were submitted to clinical examination, dermatological and neurological evaluation, neuroimaging study (CT and MRI brain) as well as neurophysiological evaluation, laboratory assessment including liver function tests, serum cryoprecipitate immune-electrophoresis, antinuclear antibody, rheumatoid factor as well as abdominal ultrasound and revision of histopathlogical findings.

Detection of Cryoglobulins

The thermolability of cryoglobulins necessitated that the blood samples be collected at 37°C. Syringes and collection tubes were at 37°C at the time of blood collection and maintained at 37°C until clotting is completed. Tubes for collection were not anticoagulated, since the use of plasma may result in the development of cold-precipitable fibrinogen (cryofibrinogen) or heparin-precipitable protein. Blood Sampling10mL of blood in a warm syringe (37°C) were collected.

Analysis Process:

A white precipitate (cryoglobulin) appeared in the serum after 24-72 hours of storage at 4°C. The serum was tested for the reversibility of the cryoprecipitate by re-warming an aliquot at 37°C for 24 hours.

The cryocrit was estimated by measuring the height of the column of precipitated protein relative to the height of the serum column[17].

Liver histology was evaluated according to the standard international criteria using Metavir Score (**Table 1**).

Statistical Methods

Frequency, mean, standard deviation and standard error of mean were used to describe data.

Mann-Whitney μ test was used to test for significance of difference in quantitative variables between each two groups.

Chi-square test was used to test for association between groups and clinical categorical data. P value was considered significant if < 0.05.

These tests were run on an IBM compatible personal computer using the Statistical Package for Social scientists (SPSS) for windows 7.5 (SPSS Inc, Chicago, IL, USA).

The study protocol was approved by human ethics committee of Specialized Medical Hospital Mansoura University.

As regard to laboratory findings including liver function tests (SGPT, serum bilirubin and prothrombin time), urine analysis (for proteinuria), serum creatinine, ANA, RF and quantitative PCR for HCV-RNA, there were no statistically significant difference between cryo +ve and cryo –ve patients except for proteinuria.

Concerning the liver histopathological findings (evaluated according to Metavir score) there were no statistically significant difference in activity score between cryo +ve versus cryo –ve patients.

Results

The results are shown in **Tables 2-5**.

Table 1: Metavir Score A0-3 (Activity) F0-4 (Fibrosis) [18]

Activity	A0 (None)	A1 (Mild)	A2 (Moderate)	A3 (Severe)	
Fibrosis	F0	F1	F2	F3	F4

Activity (A): An algorithm of both piecemeal and parenchymal necrosis
Fibrosis (F): Non, F1: Portal tract expansion by fibrosis, F2: <50% bridging fibrosis, F3: >50% bridging fibrosis including incomplete cirrhosis, F4: Established cirrhosis

Table 2: Demographic data, clinical characteristics, skin & neurological manifestations

Features	Frequency		Descriptive *(n=40)*	
	n	%	Mean	SD
Sex: female	14	35	37.53	9.16
male	26	65		
Fatigue	27	67.5		
Parasthesia	12	30		
Arthralgia	13	32.5		
Pruritis	10	25		
Edema	2	5		
Face Pigmentation	17	42.5		
Jaundice	12	30		
BMI: low	7	17.5		
average	22	55		
over	11	27.5		
Leucocytoclastic vasculitis	9	22.5		
PCT	8	20		
Lichen Planus	7	17.5		
Acral NE	6	15		
Vetiligo	6	15		
Symptomatic Neuropathy	4	10		
Myopathic	0	0		
Fits	0	0		
EMG: neuropathic	12	30		
NCV: delayed	12	30		
EEG: abnormalities	9	22.5		

Table 2 *shows the demographic details, patient clinical characteristics, skin and the neurological manifestations of the studied patients. Among 40 patients with chronic hepatitis C, there were 26 male (65%) and 14 female (35%). The main age of the patients was 37.5 years. The most common symptom was fatigue 27 cases (67.5%). Leukocytoclastic vasculitis was the main skin lesion 9 cases(22.5%) followed by porphyria cutenia tarda 8 cases (20%) and Lichen planus 7 cases (17.5%). Symptomatic neuropathy was only found in 4 cases (10%) while in 12 cases (30%) neuropathy was detected by nerve conduction velocity and EMG. EEG changes were detected in 9 cases (22.5%).*

Discussion

The present study demonstrated a high prevalence of Cg: 62.5% in patients with chronic HCV infection. Different studies showed wide variation from 19% - 57%[19-21]. These discrepancies may be caused by different Cg detection methods and a strong regional differences.

Many skin lesions were detected in the studied patients; including face pigmentation (42.5%), leukocytoclastic vasculitis (LCV) (22.5%), porphyria cutanea tarda (PCT)(20%), lichen planus (17.5%), acral necrolytic erythema (15%) and vitiligo (15%). Arthur *et al*[16] found that cutaneous symptoms or findings relevant to HCV infection manifest in 20 – 40 % of patients presenting to dermatologists and in a significant percentage (15 – 20 %) of general patients.

Neurological manifestations, including symptomatic neuropathy in 10%, neuropathic changes (demyelinating neuropathy) detected by nerve conduction velocity (NCV) and electromyography (EMG) in 30%, also electroencephalographic changes in 22.5%. Our results are in accordance with that of Zaltron *et al*[10], Ripault *et al*[13], Bonetti *et al*[22], and Arthur *et*

al[16]. These cutaneous and neurological manifestations detected in the studied patients were significantly associated with the presence of cryoglobulinaemia.

Table 3: Comparison of clinical characteristics in cryo +ve versus cryo –ve patients

Features	Cryoglobulinemia		Chi square	
	-ve	+ve	X^2	P
Fatigue	9	18	0.615	0.433
Parasthesia	3	9	1.143	0.285
Arthralgia	4	9	0.372	0.542
Pruritis	0	10	8.000	0.005
Edema	0	2	1.263	0.261
Face Pigmentation	1	16	12.610	0.000
Jaundice	5	7	0.127	0.722
BMI: low **average** **over**	6 8 1	1 14 10	10.743	0.005

Table 3 *shows comparison of clinical characteristics in cryo+ve { 25/40 (62.5%) }versus Cryo –ve patients { 15/40 (32.5%) }. Cryoglobulinaemia was detected in 25 cases (62.5%). There were no statistically significant difference in clinical characteristics between Cryo –ve and Cryo +ve cases apart from pruritis, face pigmentation and BMI.*

Table 4: Relation of skin manifestations in patients with and without cryoglobulinemia

Features	Cryoglobulinemia		Chi square	
	-ve	+ve	X^2	P
Leucocytoclastic vasculitis	0	9	6.968	0.008
Spider nevai	1	9	4.302	0.038
Palmar Erythema	0	9	6.968	0.008
PCT	0	8	6	0.014
Lichen Planus	0	7	5.091	0.024
Acral NE	0	6	4.235	0.04
Vitiligo	0	6	4.235	0.04

Table 4 *shows the relation of skin manifestations in cryoglobulinemia +ve versus Cryoglobulinamia –ve patients. Skin lesions including leucocytoclastic vasculitis, spider nevai, palmer erythema, PCT, Lichen Planus, Acral NE & Vetiligo showed statistically significant difference between cryo +ve and cryo -ve cases.*

Table 5 : Comparison of neurologic findings (clinical and neurophysiological) in Cryo+ve versus Cryo–ve patients

Features	Cryoglobulinemia		Chi aquare	
	-ve	+ve	X^2	P
Symptomatic neuropathy	0	21 4	2.667	0.102
Myopathy	0	25	-	-
Fits	0	25	-	-
EMG: Neuopathic	0	12	10.286	0.001
NCV: Delayed	0	12	10.286	0.001
EEG: Abnormal	0	9	6.968	0.008

Table 5 *showed comparison of neurologic fidings (clinical and neurophysiological) in Cryo+ve versus Cryo–ve patients.There were no statistically significant difference between Cryo+ve and Cryo–ve patients for symptomatic neuropathy while there were statistically significant difference for abnormalities detected by electrophysiological studies(EMG, NCV & EEG).*

Origgi *et al*[14] stated that cryoglobulinaemia may be one of the mechanisms (vasculitis associated with cryoglobulinaemia and or multiple ischaemic changes) underlying cutaneous and neurological manifestations associating HCV infection.

Chronic sensory polyneuropathy was the most frequent neurological manifestation, and this was the same as findings by Nemni *et al*[23] who found that the prevalence of polyneuropathy was significantly higher in cg +ve versus cg –ve patients, however, Paoletti et al[24] and Lidove et al[25] reported some HCV+ve patients with polyneuropathy and persistent negativity for cryoglobulins.

Proteinuria was present in 22.5% of cryo positive patients in the current study. Valentina *et al*[26] found that the presence of proteinuria correlated with very high cryocrit level. Other biochemical parameters and Quantitative PCR in the studied patients, showed no statistically significant difference between cryo positive and cryo negative patients. These were in accordance with Horcajada *et al* [11] and valentina *et al*[26].

Liver histopathology of the studied patients according to METAVIR score (Activity)A0-3 (Fibrosis) F0-4 showed no statistically significant difference between cryo positive versus cryo negative patients. These observations are in accordance with the data reported by Valentina *et al*[26] and Schmidt *et al*[21] who also found no correlation between the amount of Cg and histological activity.

Sansonno and Dammacco[27] reported that HCV-infected people with cryoglobulinemic vasculitis frequently show minimal liver damage and normal or slightly increased levels of serum aminotransferases.

Several authors have reported a higher prevalance of Cg in patients with HCV-induced liver cirrhosis than in chronic hepatitis C without cirrhosis; however, this observation has not been confirmed by others Lunel *et al*[28]. Also, Wong *et al*[19] reported no differences in the stage of fibrosis between patients with and without cryoglobulinaemia.

Conclusion

Our findings confirmed a clear association between cryoglobuliaemia and extrahepatic neurocutaneous manifestations of HCV infection. The presence of these manifestations in the appropriate clinical setting should suggest the presence of HCV infection and HCV antibodies should be

tested and, if positive, HCV-RNA is indicated and if there is any evidence of an etiological association of replicative HCV infection and these extrahepatic manifestations, antiviral treatment should be considered.

We are in need to extend this work to study Hepatitis C virus induced cryo globulinaemia quantitatively ; essential mixed cryoglobulinaemia (EMC), and to assess if it has any role in HCV-liver disease progression.

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